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Identification and characterization of sweetpotato ESTs highly expressed in suspension-cultured cells as well as induced the gene expression under environmental stress

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Objectives

To identify genes highly expressed in plant suspension cultures as well as induced their expression under environmental stresses, cDNA library was constructed with suspension cells of sweetpotato and 1431 single-pass sequences of the 5' ends were obtained. Suspension cell ESTs were compared with roots and leaves ESTs of sweetpotato as well as the suspension cells ESTs from other plants.

Materials and Methods

1. Material

Plant – Sweetpotato cultivar (*Ipomoea. babatas* cv. Yulmi)

Suspension culture cell lines – SP-47 cell.

2. Methods:

cDNA library was constructed using a uni-ZAP cDNA synthesis kit. For the cold stress, whole plants were incubated at 15℃ under light for 14h, and in the dark for 10h. The two and third leaves from the top were sampled 24h, 48h after cold treatment. For the MV treatment, 50μM MV solution containing 0.125% triton X-100 was sprayed onto whole plants. The two and third leaves from the top were sampled 6h, 12h, 24h after MV treatment. For H_2O_2 treatments, the two and third leaves from the top were removed from each plant and incubated in Falcon tubes containing 440mM H_2O_2 solution at 25℃ for 24h and 48h. Sterile water was used as a control for H_2O_2. All treated plant materials were immediately frozen in liquid nitrogen and stored at -70℃ until further use.

Results and Discussion

1. Production of pharmaceutical proteins and secondary metabolites in plant cultured cells has actively studied due to its potential utility. A powerful expression system with an appropriate promoter is key requisite for expression of foreign genes efficiently in cultured plant cells. To identify genes highly expressed during cell growth of cultured cells in sweetpotato, large-scale single pass cDNA sequencing was employed with cDNA clones of late exponential phase. The 1431 ESTs were generated and analyzed with BLASTX against NCBI and with roots and leaves ESTs of sweetpotato databases. Taken together above analysis, twenty two candidate ESTs were selected and then subjected into Northern analysis. All of them showed high expression in suspension cells compared with leaves. Five genes among twenty two genes were investigated to know expression pattern during growth stage of suspension cultures and gene copy number. In addition, the number of ESTs associated with the five genes was compared with that in the suspension cell ESTs from other plants.

2. It has been known that cultured plant cells are grown under conditions of high oxidative stress. Plant cultured cells were found to have much higher levels of antioxidants enzymes than differentiated plant tissues. Particularly, a sweetpotato cell lines produced a very high level of POD. Here we will show that the most abundant five ESTs in suspension cultures of sweetpotato are induced by environmental stresses. Promoter of genes highly expressed in suspension cultured cells of sweetpotato as well as oxidative stress related will be helpful for higher productivity and increased application of plant cultured cells for the production of high-value recombinant proteins.

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